

## Effects of Heat Processing and Storage on Flavanols and Sensory Qualities of Green Tea Beverage

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This research was conducted to understand the effects of heat processing and storage on flavanols and sensory qualities of green tea extract. Fresh tea leaves were processed into steamed and roasted green teas by commercial methods and then extracted with hot water (80 °C) at 1:160 ratio (tea leaves/water by weight). Green tea extracts were heat processed at 121 °C for 1 min and then stored at 50 °C to accelerate chemical reactions. Changes in flavanol composition and sensory qualities of green tea extracts during processing and storage were measured. Eight major flavanols (catechin, epicatechin, gallic catechin, epigallocatechin, epicatechin gallate, catechin gallate, epigallocatechin gallate, and gallic catechin gallate) were identified in the processed tea extract. Among them, epigallocatechin gallate and epigallocatechin appeared to play the key role in the changes of sensory qualities of processed green tea beverage. The steamed tea leaves produced a more desirable quality of processed green tea beverage than the roasted ones.

**Keywords:** Green tea extract; heat processing; storage; flavanols; sensory qualities

### INTRODUCTION

Tea extract is one of the most popular beverages in the world, and it is recognized for its high content of polyphenols, in particular, flavanols (flavan-3-ols or catechins). The total content of polyphenols in tea flush is 20–35% on a dry weight basis, and up to 90% of the polyphenols is composed of flavanols (Yamanishi et al., 1995). Flavanols have recently received much attention due to their pharmaceutical functions, such as antioxidative, antitumor, and anticarcinogenic activities (Sakanaka et al., 1989; Matsuzaki et al., 1985; Conney et al., 1992; Wang et al., 1992; Chung, F., et al., 1992; Prochaska et al., 1992; Chung, K., et al., 1998). Among the three main types of commercial teas, green tea is a kind of unfermented tea that preserves a higher quantity of flavanols than oolong (semifermented) and black (fermented) teas (Terada et al., 1987). Assessed by various experimental methods, green tea does show higher antioxidative activity than other semifermented or fermented teas (von Gadov et al., 1997). In view of the pharmaceutical functions of flavanols, green tea is a very good choice for consumers.

For several years, manufacturers have been trying to package green tea extract in cans or glass bottles that can be dispensed in vending machines in order to increase the consumption of green tea and to meet changing lifestyles. A suitable product of green tea extract is presumed to have a balanced taste of bitterness, tangy astringency, and a persistent sweet after-taste (Sanderson et al., 1976). Also, the color of green tea extract, clear, greenish-yellow, without any trace of

red or brown color, has been judged to be very appealing (Wickremasinghe, 1978). However, it was noted that the production of green tea extract in cans was more problematic than that of black or oolong tea extracts (Yamanishi et al., 1995). Green tea extract with its distinctive, delicate sensory characters does not blend well with many additives, such as sugars, lemon, or milk and, as a result, is usually consumed plain. It becomes difficult to refine the taste once the important sensory attributes in green tea extract are lost. Presently, "canned" green tea beverage has not been popular in the soft drink market because of the loss of these sensory qualities of green tea extract during processing and storage. Because canned green tea products are relatively new and there has been only limited information on processed green tea extract available, the objective of this research is to determine the effects of heat processing and storage on the flavanol composition and sensory qualities of green tea extract.

### MATERIALS AND METHODS

Fresh tea leaves (*Camellia sinensis*) harvested in August 1998 at Posung, Korea, were immediately processed into commercial steamed and roasted green teas. The steamed tea leaves were first "fixed" by steam at 95–100 °C for 15–30 s to inactivate enzymes and then dried in four steps of heating (70–80 °C for 35–40 min, 60–70 °C for 30–40 min, 80–90 °C for 15–20 min, and 60–75 °C for 30–40 min) until the moisture content of the final product reached 3.82%. For the manufacture of roasted tea, fresh tea leaves were first withered and fixed by pan firing (160–230 °C for 10–12 min) and then also gradually dried by heating (150 °C for 10–15 min, 110 °C for 15–20 min, 100 °C for 10–15 min, and 80–90 °C for 30 min). The moisture content of the final roasted tea product was 3.29%. The processed tea leaves were sieved (No. 18, 1 mm), vacuum packaged in polypropylene (PP) plastic bags, and then stored in a 0 °C storage room. One day prior to use, bags of tea leaves were removed from the storage room and kept in desiccators at room temperature overnight. To avoid the problem of tea sample variations in our research, all experiments were

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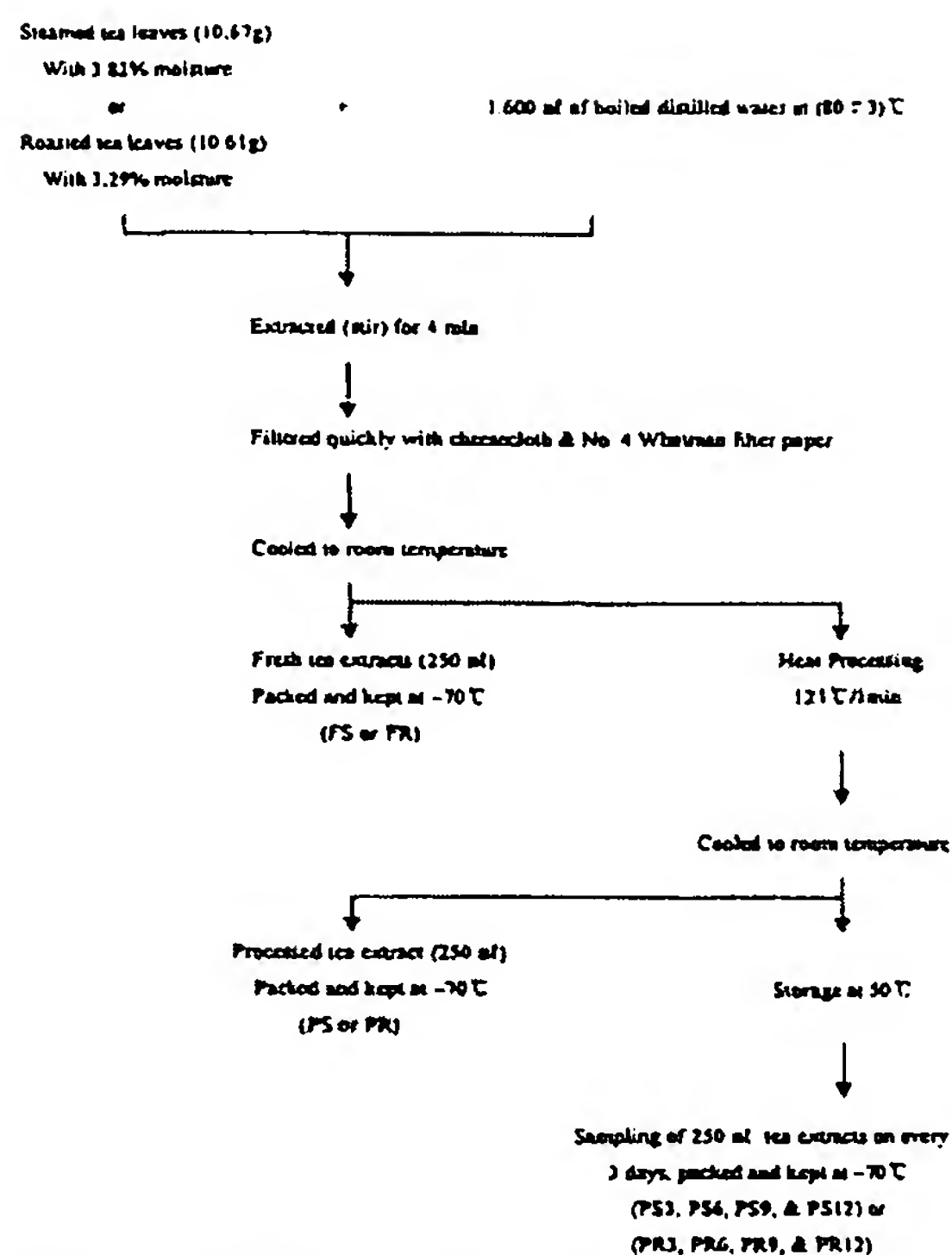


Figure 1. Preparation of fresh, processed, and stored green tea extracts. Abbreviations: FS (FR), freshly prepared green tea extracts made from steamed (roasted) tea leaves; PS (PR), heat-processed steamed (roasted) tea extracts; PS3, PS6, PS9, and PS12 (PR3, PR6, PR9, and PR12), heat-processed and stored (for 3, 6, 9, and 12 days at 50 °C) tea extracts prepared from steamed (roasted) tea leaves.

conducted on samples from the same brew that were steamed or roasted on the same day.

**Sample Preparation.** Green tea extracts were prepared according to a method modified from the manufacture of Japanese canned green tea extract (Suematsu et al., 1992), as shown in Figure 1. Fresh tea extracts in glass jars were heat processed at 121 °C for 1 min and then stored at 50 °C for up to 12 days to accelerate chemical reactions simulating a 70-day period of storage at 20 °C (Zimeri et al., 1999). According to the preparation procedure, samples of steamed tea extracts were designated FS (freshly prepared steamed green tea extract), PS (heat-processed steamed tea extract), and PS3, PS6, PS9, and PS12 (heat-processed steamed tea extracts that were stored at 50 °C for 3, 6, 9, and 12 days), respectively. Similarly, samples of roasted tea extracts were designated FR, PR, PR3, PR6, PR9, and PR12, respectively. After treatments, all samples were transferred to plastic bags, sealed, and kept in a -70 °C freezer until analyzed.

**Measurement of Color.** The color of fresh and processed tea extracts was measured using a HunterLab color meter (ColorQuest II, Hunter Associates Laboratory, Inc., Reston, VA).

**Assessment of Taste Quality.** Sensory evaluation was carried out in a light-masked room to avoid the influence of sample colors on the panelist's decisions. Twelve qualified panelists were selected from 32 volunteers by a triplicated pretest in which four concentrations of steamed tea extract (tea leaves/water = 1:80, 1:100, 1:120, and 1:160) were tasted. Questionnaires, based on the method of magnitude estimation of descriptive analysis, were composed of lines of 15 cm—the

highest intensity was expressed as 15 and the lowest as 0. The results of triplication were analyzed using ANOVA for multiple comparison (SAS/STAT software, SAS Institute Inc., Cary, NC).

**Assay of Total Phenolic Content.** The content of total phenolics was measured according to the Folin–Ciocalteu assay (Slinkard et al., 1977) spectrophotometrically at 765 nm (Hewlett-Packard spectrophotometer model 8452A, Rockville, MD). Samples of green tea extracts were diluted 5 times for assay. The total phenolic content of tea extracts was calculated from the calibration curve prepared from the absorbance of gallic acid standard solutions.

**Analysis of Flavanol Compounds.** Samples of green tea extracts were pretreated according to the method described by Matsuzaki et al. (1985). Samples of 10 mL of green tea extracts were washed three times using the same volume of chloroform and then extracted three times with a total of 30 mL of ethyl acetate. The ethyl acetate layers were combined, vacuum evaporated, dissolved in 10 mL of 50% acetonitrile solution (HPLC graded), and filtered through a 0.45 µm Acrodisc 13 CR PTEE filter (Gelman Sciences, Ann Arbor, MI) for HPLC analysis. Authentic reagents of (+)-catechin, (-)-epicatechin (EC), (-)-gallocatechin (GC), (-)-epigallocatechin (EGC), (-)-epicatechin gallate (ECG), (-)-catechin gallate (CG), (-)-epigallocatechin gallate (EGCG), and (-)-gallocatechin gallate (CGG) were purchased from Sigma (St. Louis, MO) and prepared at the concentration of 0.1 mg/mL in 50% acetonitrile solution to be used as HPLC references. It has to be noted that these artificial authentic reagents may possess different optical rotation properties from the ones existing in the natural tea flush. For example, gallocatechin found in the fresh tea flush is in the positive form (Yamanish et al., 1995), but the gallocatechin available from Sigma was in the negative form. However, this did not influence the identification of flavanols in this experiment, because the HPLC method described below could not separate the same flavanols of various optical rotation properties. Also, the optical rotation of flavanols in the heat-processed tea leaves or tea extracts may be in an impure status due to the "isomerization" of flavanols. (This will be explained under Results and Discussion.) Therefore, the (+) or (-) optical rotation property for each flavanol is not indicated in this paper.

Conditions of HPLC analysis modified from those of Ikegaya (1985) and Ikegaya et al. (1990) were adopted. The references and pretreated samples were analyzed using an HPLC system (Jasco Trirotar VI) connected with a UV-vis detector (ATTO SF-1205) set at 280 nm. The Waters µ-Bondapak C<sub>18</sub> pre-packed column (3.9 × 300 mm) was pre-equilibrated with a mobile phase consisting of acetonitrile, ethyl acetate, and 0.05% phosphoric acid aqueous solution [120:20:860 (v/v/v)] at a flow rate of 1.2 mL/min and at the temperature of 40 °C. After the back pressure was stabilized at ~67 kgw/cm<sup>2</sup>, 5 µL of reference or sample was injected into the column and eluted with the mobile phase. Identification and quantification of flavanols were achieved by comparing retention times and peak area on the chromatograms with the references.

## RESULTS AND DISCUSSION

**Changes in Sensory Qualities of Green Tea Extracts.** Changes in color of green tea extracts during processing and storage were expressed as *L*, *a*, and *b* values. A decrease in *L* value and increases in *a* and *b* values indicate the development of a brown color. Both steamed and roasted tea extracts gradually turned dark during processing and storage, and their *L* values steadily decreased from the range of 87–90 (FS and FR) to 65–67 after processing and 12 days of storage (PS12 and PR12). Redness (*a* value) of both steamed and roasted tea extracts also gradually increased in a similar fashion during processing and storage, from -3, -4 (FS and FR) to +9–11 (PS12 and PR12). However, there was a large difference in yellowness between steamed



**Table 1. Effect of Processing on Taste Quality of Steamed and Roasted Tea Extracts<sup>a</sup>**

	FS	PS	FR	PR
bitterness	9.753a	9.524a	7.878b	7.465b
astringency	9.297a	9.241a	8.276ab	7.582b
sweetness	3.644a	3.711a	4.368ab	4.489b

<sup>a</sup> There is no significant difference at the level of 0.05 ( $\alpha$  value) among samples with the same letters.

**Table 2. Sensory Evaluation of Processed Steamed Tea Extract during Storage<sup>a</sup>**

	PS	PS3	PS6	PS9	PS12
bitterness	9.297a	7.903ab	6.962bc	6.091bc	5.854c
astringency	9.067a	7.983ab	6.828b	6.822b	6.451b
sweetness	3.570a	4.150ab	4.713abc	5.568bc	6.324c

<sup>a</sup> There is no significant difference at the level of 0.05 ( $\alpha$  value) among samples with the same letters.

**Table 3. Sensory Evaluation of Processed Roasted Tea Extract during Storage<sup>a</sup>**

	PR	PR3	PR6	PR9	PR12
bitterness	7.546a	5.502b	5.074b	4.720b	3.790b
astringency	7.739a	5.463b	5.364b	5.024b	3.928b
sweetness	4.491a	4.868ab	5.626ab	6.260b	6.817b

<sup>a</sup> There is no significant difference at the level of 0.05 ( $\alpha$  value) among samples with the same letters.

and roasted tea extracts during processing. Roasted tea extract showed an increase in *b* value from 14 (FR) to 33 (PR) during processing, whereas steamed tea extract increased its *b* value from 13 (FS) to only 24 (PS). Even though the difference in yellowness became smaller with prolonged storage time, and no significant differences in color were present at the final stage of storage between steamed and roasted tea extracts (PS12 and PR12), the roasted tea extract was more prone to color changes with heat treatment.

As mentioned previously, the taste quality of green tea extract consists of three major characteristics: bitterness, astringency, and a persistent sweet after-taste. Table 1 shows the sensory qualities of fresh and processed green tea extracts. There were no statistical differences between the fresh and processed green tea extracts, even though the average data show that fresh tea extracts (FS and FR) were slightly more bitter and astringent and less sweet than their processed extracts (PS and PR). Comparisons between steamed and roasted tea extracts showed that the steamed tea extract tasted significantly more bitter, more astringent, and less sweet than the roasted one. Tables 2 and 3 show the taste qualities of processed steamed and roasted tea extracts during storage, respectively. Both processed tea extracts changed in the same fashion, becoming less bitter, less astringent, and sweeter in their tastes during storage. On the basis of these results, it is conceivable that steamed tea leaves would result in a better canned product.

**Changes in Total Phenolic Content of Green Tea Extracts.** The contents of four free sugars (fructose, glucose, sucrose, and maltose) and total free amino nitrogen of green tea extracts in this research have also been analyzed using HPLC and ninhydrin assay, respectively. However, neither free sugar contents nor total free amino nitrogen changed corresponding to the color changes of green tea extracts, and the Maillard reaction appeared to be not an important factor for the browning of green tea extracts during processing and storage. Another possible reason for the browning of

green tea extracts is the oxidation of phenolic compounds, and this theory could be proven by evaluating the changes of total phenolic content in green tea extracts using the Folin–Ciocalteu test. The result of the Folin–Ciocalteu assay showed that the fresh steamed tea extract contained more phenolic compounds than the fresh roasted extract, originally. During processing, the total phenolic contents of steamed and roasted tea extracts decreased by 8.43 and 11.46%, respectively, and continuously decreased during storage. At the final storage period (the 12th day), the total phenolic content of steamed tea extract was still slightly higher than the roasted. These observations reflected the decrease in bitterness and astringency of sensory evaluation during processing and storage, and the steamed tea extract had a stronger taste of bitterness and astringency than the roasted one. Even though caffeine has been reported to be an important source of bitter taste in tea extracts (Oh et al., 1988), the result of our caffeine analysis by HPLC showed that the content of caffeine remained quite stable and did not correlate with the taste changes of green tea extracts during processing and storage. Therefore, phenolic compounds were judged to be the key elements that determine both color and taste qualities of green tea beverage during processing and storage. Additionally, the Folin–Ciocalteu assay is a redox method based on the ability of phenolics to react with oxidizing agents. Usually, redox assays are affected by the varying hydroxylation pattern and the degree of polymerization of polyphenolics. The decreases in total phenolic content were mainly due to the fact that the phenolic compounds in green tea extracts were oxidized or polymerized during processing and storage.

**Changes in Flavanols of Green Tea Extracts.** Figure 2 shows the HPLC chromatograms of flavanols in steamed tea extracts. (The chromatograms of roasted tea flavanols were similar to those of the steamed.) Originally, seven flavanols were detected in the fresh green tea extracts (FS and FR), and they were identified as GC, EGC, catechin, EC, EGCG, GCG, and ECG, in order from left to right on the HPLC chromatograms, by comparing their retention times with those of the authentic standards. After heat processing, the amounts of GC, catechin, and GCG were significantly increased, and another flavanol, CG, appeared after the ECG peak. During 12 days of storage, all flavanols gradually decreased, and the decrease in EGCG was the most significant.

Quantification of flavanols was performed by comparing their peak area of the HPLC chromatograms with the standards, and the result is shown in Table 4. Originally, there were about 0.65 mg of EGCG/mL, 0.22 mg of EGC/mL, 0.13 mg of ECG/mL, 0.08 mg of EC/mL, 0.05 mg of catechin/mL, and trace amounts of GCG and GC in the fresh green tea extracts. As freshly prepared samples, steamed tea extract (FS) contained more flavanols than the roasted (FR), and the difference in EGCG could reach 0.2 mg/mL. Looking into the processing methods of steamed and roasted tea leaves, roasted tea leaves were "fixed" with a much higher temperature than the steamed. During the high-temperature fixing, more flavanols in the roasted tea leaves could be lost.

In addition, green tea flavanols have been classified, according to their stereochemical configurations, into "catechins" (2,3-trans, such as catechin, CG, GC, and GCG) and "epicatechins" (2,3-cis, including EC, ECG,

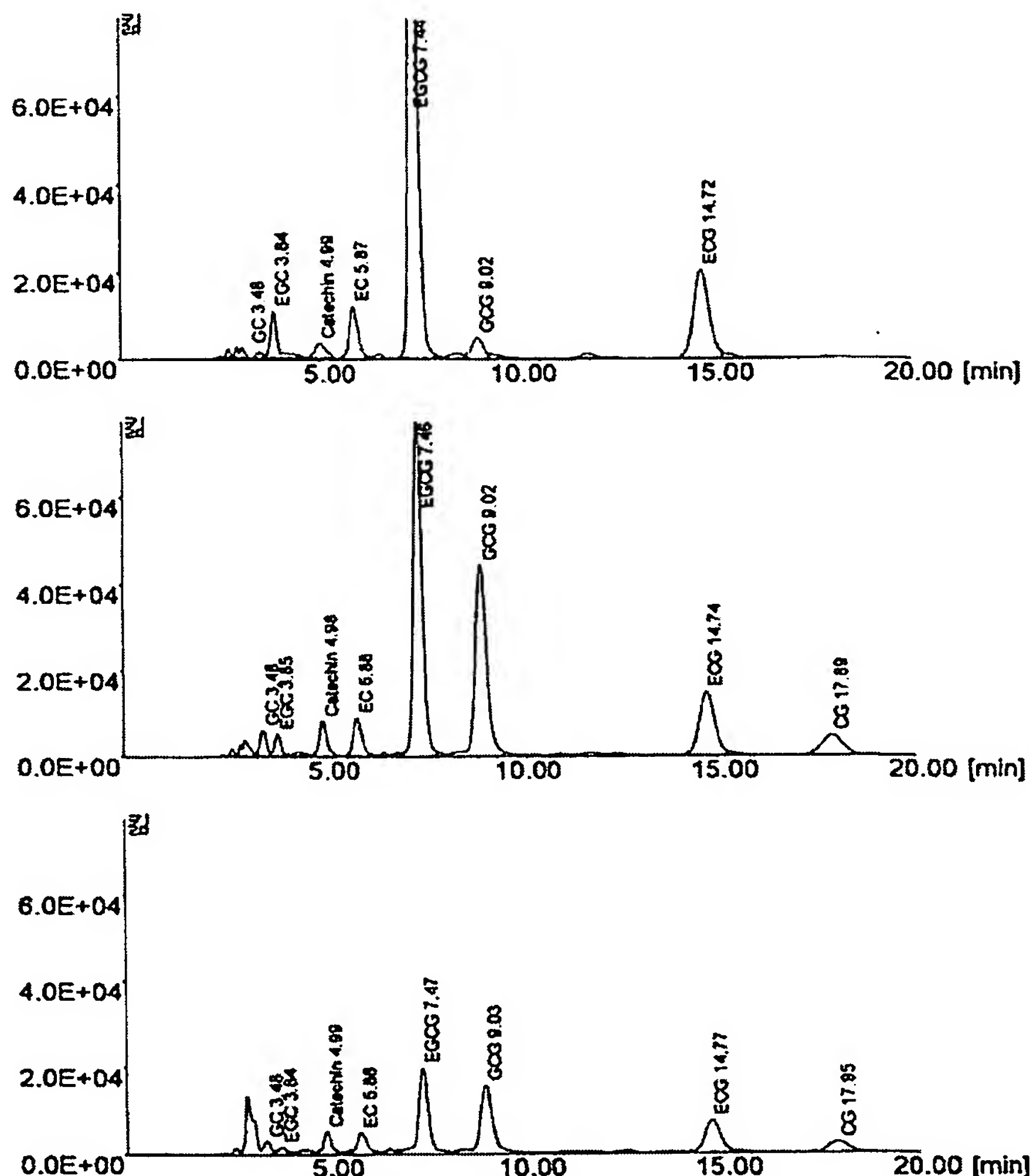


Figure 2. HPLC chromatograms of flavanols in steamed green tea extract during processing and storage: (top) FS; (middle) PS; (bottom) PS12.

EGC, and ECGG) (Figure 3). Epicatechins of both steamed and roasted tea extracts decreased steadily during processing and storage, whereas catechins were first increased by heat processing and then decreased during storage. The increase of catechins was believed to result from the "isomerization" of epicatechins. It has been reported that green tea flavanols undergo isomerization by high-temperature treatment, which involves a change in configuration at the C-2 position without changing the optical rotation (Seto et al., 1997). For example, (-)-EC isomerizes to (-)-catechin, and (+)-catechin isomerizes to (+)-EC. Both epicatechins and catechins could isomerize under our processing conditions, and we have proved this by individually adding the same amount of isomers (e.g., EC and catechin) into the green tea extracts, which were then processed and stored as described previously. The addition of EC caused a rapid increase of catechin, and the addition of catechin also resulted in a lower loss of EC than the

control sample. Inferred from their stereochemical configuration, catechins with their "2,3-trans" structure are thermodynamically more stable than epicatechins, which are "2,3-cis", so catechins isomerized at a lower rate than epicatechins, resulting in the different equilibria during the heat treatment, expressed as



This inference can reasonably explain why the concentrations of catechins in green tea extracts were increased by heat processing.

Quantitatively, epicatechins are much more important than catechins in deciding the qualities of green tea extracts, because >90% of the flavanols in green tea extracts consist of epicatechins. Figure 4 shows the loss percentages of various epicatechins, on the basis of their original amounts in FS and FR, during processing and

Table 4. Changes of Flavanols in (A) Steamed and (B) Roasted Tea Extracts during Processing and Storage

sample	flavanol <sup>a</sup>								total
	GC	EGC	catechin	EC	EGCG	GCG	ECG	CG	
(A) Flavanol Contents in Steamed Tea Extract (mg/mL)									
FS	0.0076	0.2401	0.0579	0.0912	0.7526	0.0098	0.1452	0	1.3044
PS	0.0115	0.1928	0.0709	0.0682	0.4844	0.1396	0.1269	0.0128	1.1065
PS3	0.0092	0.1082	0.0660	0.0536	0.4085	0.1235	0.1061	0.0109	0.8869
PS6	0.0065	0.0734	0.0435	0.0414	0.2204	0.0717	0.0642	0.0069	0.5280
PS9	0.0048	0.0484	0.0392	0.0380	0.1560	0.0549	0.0574	0.0065	0.4052
PS12	0.0036	0.0415	0.0383	0.0355	0.1078	0.0432	0.0539	0.0065	0.3283
(B) Flavanol Contents in Roasted Tea Extract (mg/mL)									
FR	0.0054	0.2021	0.0361	0.0746	0.5474	0.0097	0.1188	0	0.9941
PR	0.0096	0.1138	0.0522	0.0480	0.2570	0.0725	0.0712	0.0069	0.6312
PR3	0.0071	0.1066	0.0516	0.0476	0.2183	0.0663	0.0651	0.0068	0.5694
PR6	0.0062	0.0754	0.0445	0.0426	0.1782	0.0594	0.0634	0.0067	0.4764
PR9	0.0052	0.0574	0.0415	0.0386	0.1183	0.0431	0.0566	0.0063	0.3670
PR12	0.0035	0.0491	0.0377	0.0351	0.0717	0.0292	0.0462	0.0053	0.2779

<sup>a</sup> Data are represented in the order of their positions on the HPLC chromatogram.

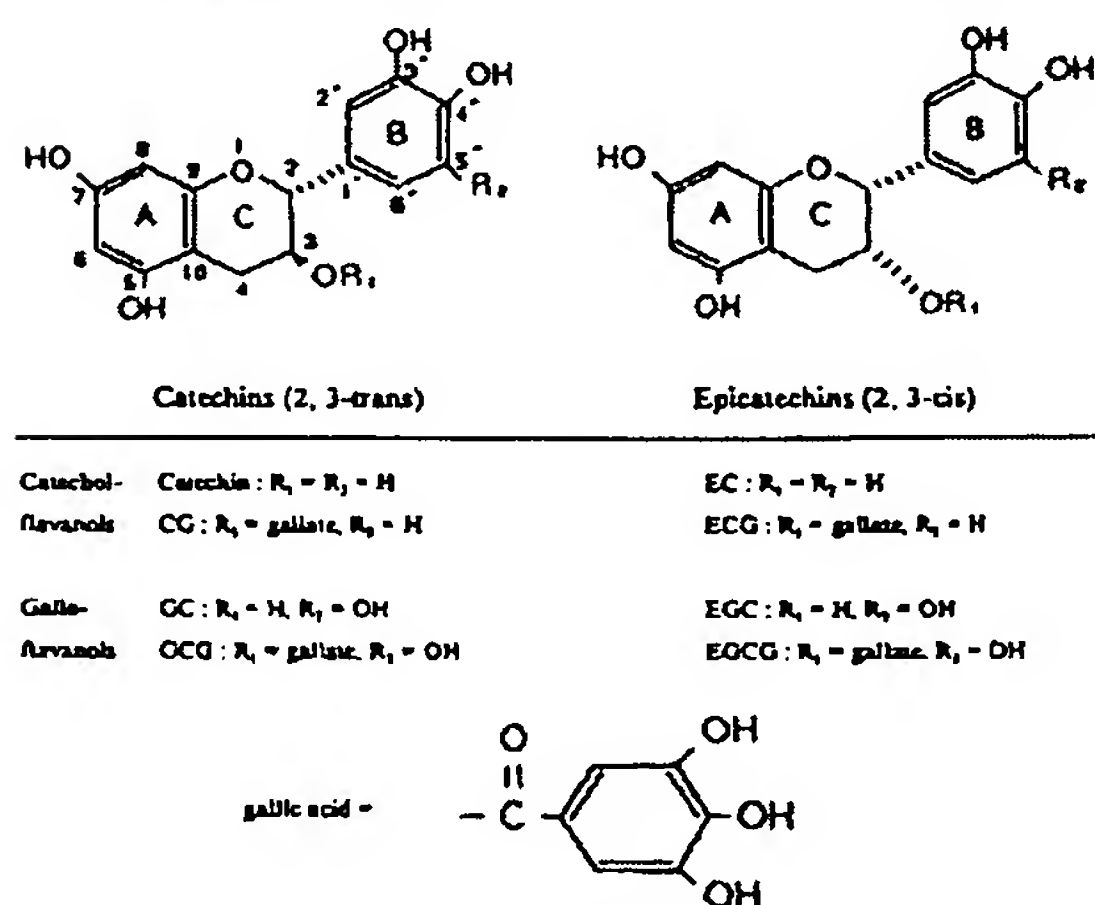


Figure 3. Classification of green tea flavanols.

storage. After processing and 12 days of storage, about 86% of EGCG, 79% of EGC, 62% of ECG, and 57% of EC in green tea extracts were lost. EGCG and EGC are not only more dominating in amounts (together they accounted for 80% of the total epicatechins) but also more unstable to oxidation. As shown in Figure 3, green tea flavanols can be also divided into "catechol-flavanols" and "gallo-flavanols", according to the number of hydroxyl groups attached to the B rings. With three hydroxyl groups on their B rings, EGCG and EGC are classified as gallo-flavanols, whereas ECG and EC, which have B rings with only two hydroxyl groups, belong to catechol-flavanols. During the oxidation process, flavanols lose one hydrogen radical and form a semiquinone radical with an unpaired electron on the oxygen atom (Figure 5). On the basis of electron spin resonance (ESR) measurement, it was suggested that the radical can form more freely on a ring possessing three hydroxyl groups than on a ring possessing two groups (Yoshioka et al., 1991). This may explain why gallo-flavanols have lower redox potentials (Opie et al., 1993) and oxidized more rapidly than catechol-flavanols during the processing and storage of green tea extracts. On the other hand, EGCG and EGC contain another ring with three hydroxyl groups in their galloyl ester function. The B rings of EGCG and EGC have been named the "galloyl moiety", and the gallic acid groups of

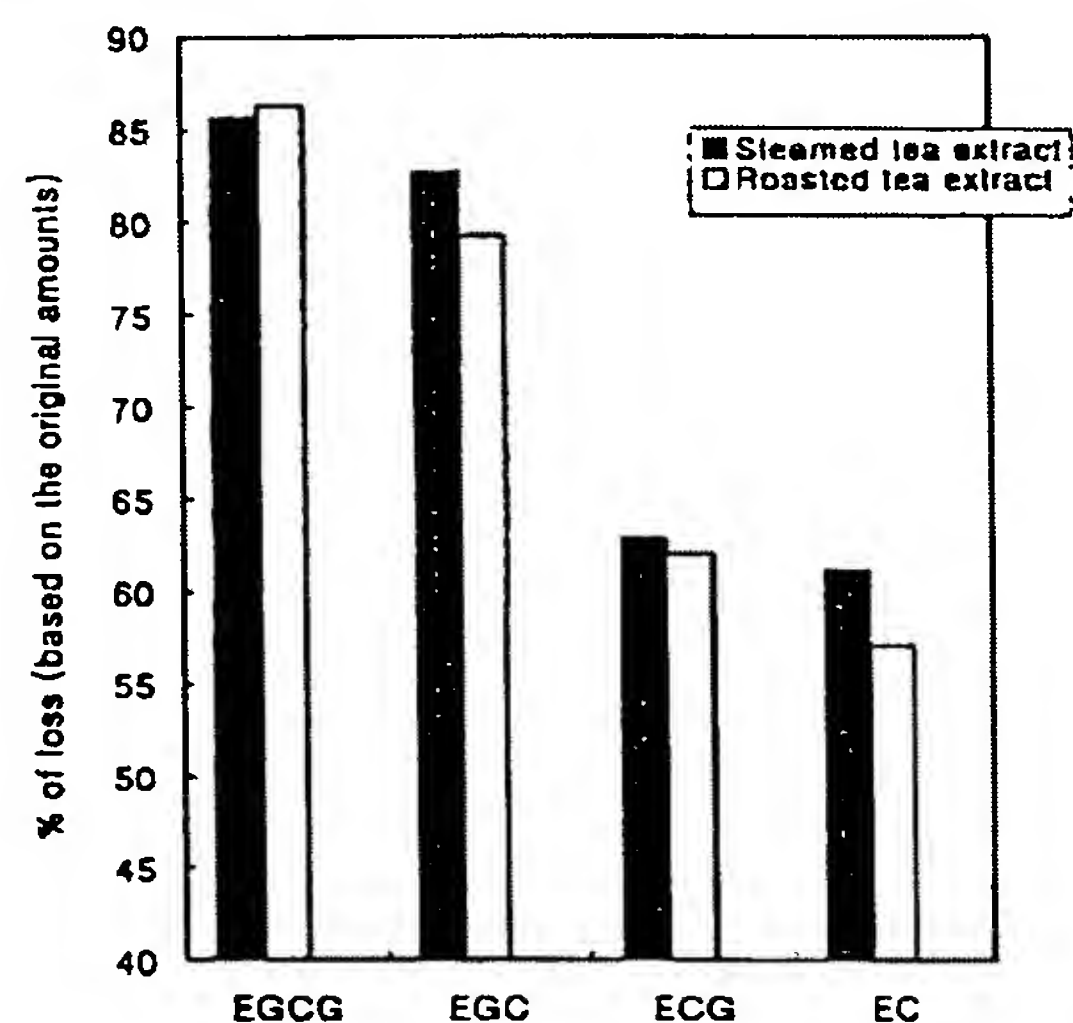


Figure 4. Decreases of various epicatechins (EGCG, EGC, ECG, and EC) in steamed and roasted tea extracts by processing and storage.

EGCG and EGC have been called the "galloyl moiety" (Yoshioka et al., 1991). Both galloyl and galloyl moieties possess three hydroxyl groups and form radicals during oxidation. The biggest difference between galloyl and galloyl groups is that there is a proton, "Hb", on the C-2 carbon in conjunction with the C-1' carbon of galloyl moiety (but Hb is not part of the galloyl moiety), as shown in Figure 5. Unlike the hydrogen atoms "Ha" located on C-2' and C-6', Hb lies in a different plane from the one with the semiquinone radical. Owing to the influence of Hb on the transformation of the B-ring oxygen radical, the galloyl moiety oxidizes more easily than the galloyl moiety, according to the ESR measurement (Yoshioka et al., 1991). In fact, the tendency to oxidize for epicatechins has been revealed in the order EGCG > EGC > ECG > EC (Yoshioka et al., 1991; Miyazawa et al., 1998), and this agrees well with our observation in this experiment. On the other hand, flavanols are well-known as effective radical scavengers by donating hydrogen radicals and reacting with alkoxyl (RO<sup>•</sup>) or peroxy (ROO<sup>•</sup>) radicals, converting them to more stable products. Therefore, flavanols have been recognized for their antioxidant activity. The antioxidant activity of



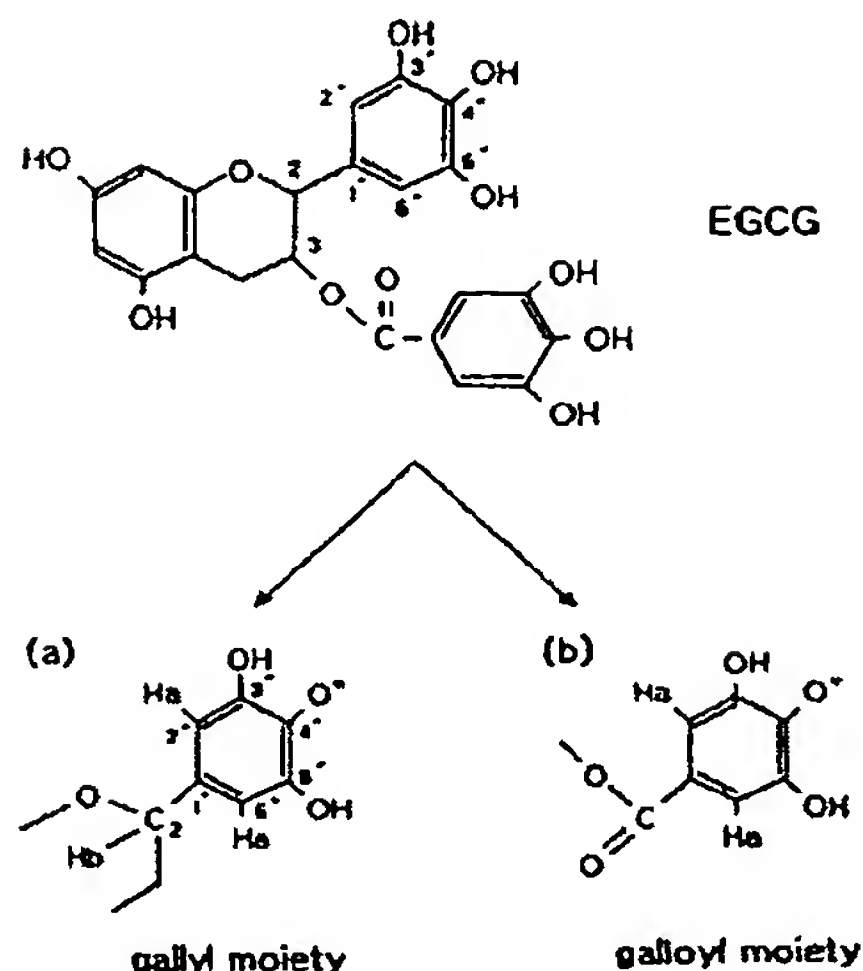


Figure 5. Formation of gallyl and galloyl radicals from EGCG. Flavanols is related to their hydrogen-donating ability. The lower redox potential and the easier formation of radicals indicate the higher hydrogen-donating ability of antioxidants. Correlated well with this principle, the antioxidant activity of flavanols was reported to increase on a molar basis: EC < ECG < EGCG (Matsuzaki et al., 1985). In view of the pharmaceutical functions of green tea, EGCG and ECG are also the most important flavanols in green tea extracts.

**Conclusion.** Flavanols, especially EGCG and ECG, appeared to play a key role in the changes of both color and taste qualities of green tea extracts during heat processing and storage. The brown color and smoothened taste of green tea extracts were probably a result of the flavanol oxidation, as determined by the assay of total phenolic content and HPLC analysis of flavanols. Additionally, steamed tea appeared to be a better choice than roasted tea for the manufacture of canned or bottled beverage, because the steamed tea extract was more stable to color and taste changes than the roasted tea. In particular, it contained more flavanols, which are important for the desirable sensory qualities of green tea extracts.

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